

# Ecology and Management of Crop Pollination and Pest Control

Insights from Red Clover Seed Production

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# Ecology and Management of Crop Pollination and Pest Control. Insights from Red Clover Seed Production

## Abstract

The agricultural landscape has gone through large changes to meet increasing demands for food. This has led to major biodiversity declines, while effects on ecosystem services that support agricultural productivity, such as pollination and pest control, remain less studied. This thesis examines temporal trends, impacts, and management for functionally important insects in agriculture using red clover seed production as a model system. Red clover is pollinated by bumble bees (*Bombus* spp.) and honey bees. Major yield losses are caused by seed weevils (*Apion* spp.), which in turn are attacked by natural enemy parasitoids. Field studies, where sites previously sampled in the 1930s-1960s were revisited 2008-2011, showed a shift towards more species poor bumble bee communities in red clover fields dominated by a few species which are less effective pollinators. Moreover, crop pest damage by seed weevils had more than doubled, while parasitism rates provided by natural enemies had decreased. In parallel to these changes, seed yields have declined and become more variable in recent decades. We performed an experiment which showed that the gain in seed set obtained when simultaneously increasing pollination and pest control outweighed the sum of seed set gains obtained when increasing each service separately. With the field data collected we also developed integrated pest management by validating a pest sampling method, developing a threshold for insecticide treatment, and suggesting minimum effective chemical pest control. We further found that pest damage was higher in landscapes with a high proportion of agricultural land, and that maximising the distance to a clover field in the previous year could function as a proactive method to decrease pest abundance. In summary, this thesis shows that agricultural intensification can jeopardise the supply of crop pollination and pest control services, and that such changes may translate into crop yield effects. It further highlights that interactions between pollination and pest control can alter the benefits obtained from service-providing organisms, and this needs to be considered to properly manage multiple ecosystem services. Simultaneous enhancement of beneficial organisms which contribute to crop productivity and integrated management of pests that pose a threat to production can contribute to sustainable food production in agriculture.

*Keywords:* *Apion* spp., biological control, *Bombus* spp., ecosystem service, integrated pest management, *Trifolium pratense*, red clover

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## List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Bommarco, R., Lundin, O., Smith, H.G. & Rundlöf, M. 2012. Drastic historic shifts in bumble bee community composition in Sweden. *Proceedings of the Royal Society - Series B* (279), 309-315.
- II Lundin, O., Rundlöf, M., Smith, H.G. & Bommarco, R. 2012. Towards integrated pest management in red clover seed production. *Journal of Economic Entomology* (105), 1620-1628.
- III Lundin, O., Rundlöf, M., Smith, H.G. & Bommarco, R. Patterns of crop pest damage in time and space: climate, landscape composition or local field management? (manuscript).
- IV Lundin, O., Smith, H.G., Rundlöf, M. & Bommarco, R. 2013. When ecosystem services interact: crop pollination benefits depend on the level of pest control. *Proceedings of the Royal Society - Series B* (280), 20122243.

Papers I, II, and IV are reproduced with the permission of the publishers.

The contribution of Ola Lundin to the papers included in this thesis was as follows:

- I Participated in the development of research questions and design. Searched for historical data in the literature and did field work in Uppland. Performed rarefaction analyses and simulations of species trait compositions. Participated in the writing of the paper which was led by RB.
- II Developed research questions and design together with supervisors. Supervised and participated in field work and lab work together with MR. Did the statistical analyses and wrote the paper with assistance from supervisors.
- III Developed research questions and design. Searched and compiled historic data, and supervised or performed field work and lab work. Did the statistical analyses and wrote the paper with some assistance from supervisors.
- IV Developed research questions and designed the study together with supervisors. Supervised or performed all field work and lab work. Did the statistical analyses and wrote the paper with some assistance from supervisors.



# 1 Introduction

Continued growth and increased wealth of the human population is projected to double the global food demand between 2005 and 2050 (Tilman *et al.*, 2011). The agricultural landscape has gone through large changes as a result of land use conversion and intensification (Robinson & Sutherland, 2002; Hoekstra *et al.*, 2005). This has led to loss and fragmentation of habitat and decrease in habitat quality for many species. It is important to conserve biodiversity for reasons which are ethical (we have no right to extirpate species), cultural (biodiversity is a part of our heritage) and utilitarian (biodiversity is useful to us), and this has led to political agreements to safeguard biodiversity (Naeem *et al.*, 2009; Perrings *et al.*, 2011). Intensified agriculture on an increasing proportion of the land surface has been identified as one of the key drivers for biodiversity loss (MEA, 2005).

Biodiversity declines might have negative feedbacks for productivity in agricultural landscapes, but so far this has been less studied. Especially temporal data on functionally important biodiversity such as crop pollinators, pests and natural enemies is scarce, and more true links between the community composition and dynamics of those organisms and the actual contribution to crop yields are needed. More knowledge is also needed on how functionally important biodiversity can be managed in modern agricultural cropping systems.



## 2 Thesis aims

The aims of this thesis are:

- to investigate temporal trends for functionally important insects in agriculture: crop pollinators, pests, and natural enemies to pests (paper I,III)
- to explore the role of these insects for crop yield (paper I-II, IV)
- to develop sustainable management options for pest control (paper II-III)

Throughout the thesis red clover seed production is used as a model system to explore these questions.



## 3 Background

### 3.1 Theory for biodiversity and ecosystem services in agricultural landscapes

Are there any theories in ecology that are universal enough to make precise and yet broadly applicable predictions about biodiversity patterns in an agricultural landscape? Are there some that at least can give guidance? In an effort to unify different general theoretical frameworks of ecology such as continuum theory, neutral theory and meta-population theory, McGill (2010) pointed out that many theories share common rules that are sufficient to reproduce many commonly observed biodiversity patterns. These are (1) that individuals within a species aggregate (occur clumped) in space, (2) that some species dominate over others in abundance, with many rare species and a few common ones, and (3) that individuals of different species roughly occur independent from each other. A limitation for prediction is that there are as yet no rules included for the drivers of species abundances and species richness in the framework (McGill, 2010).

Slightly more detailed predictions can be derived from meta-population theory (Levins, 1969; Hanski, 1999) and its subsequent development into meta-community theory (Leibold *et al.*, 2004). Meta-population models predict an importance of both local and landscape level dynamics in determining the abundance of a species in a given habitat patch in the landscape (Bengtsson *et al.*, 2003). The relative importance of local factors *vs.* landscape context depends on the strength of local dynamics relative to the role of dispersal in shaping the local community (the meta-community paradigm, Leibold *et al.*, 2004). Meta-community theory predicts that landscape heterogeneity and connectivity between habitat patches will promote species diversity, at least up to a certain point, while landscape homogenisation and intensification will favour a few generalist species with high dispersal abilities at the cost of many

other species, creating a “weedy” world (Bengtsson, 2010). When studying the impacts of land use change on mobile organisms such as insects it is evident from both theory and empirical studies that both local and landscape factors need to be considered (Tschardt *et al.*, 2012a). Consequently, both local and landscape perspectives are incorporated into this thesis.

Ecosystem services are the benefits people obtain from ecosystems. They are often classified into four groups (MEA, 2005): (1) provisioning services which are products or goods we obtain from ecosystems such as food and timber, (2) regulating services which are benefits from regulating of ecosystem processes such as water and air purification, pollination and pest control, (3) cultural services which are non-material benefits people obtain from ecosystems such as recreation, educational and spiritual values, and finally (4) supporting services such as nutrient cycling and primary production which underpin all other services. Although being valuable and much used, this classification scheme has been criticised for failing to account for important hierarchies among ecosystem services (Wallace, 2007). A definition which separate final services (e.g. food) from intermediate services (e.g. pollination which is contributing to final services) aids management and prevents double-counting of services (Fisher *et al.*, 2009).

A much explored research question has been to examine how biodiversity, primarily species richness, relates to ecosystem functioning and the provision of ecosystem services. This research has shown that there is often a positive but saturating relationship between the number of species in a community and the level of provision of the focal ecosystem function, that diversity often promotes stability in the provision of an ecosystem function, and that typically more species will be needed to support multiple ecosystem functions (Ives & Carpenter, 2007; Gamfeldt *et al.*, 2008; Cardinale *et al.*, 2012). The mechanisms responsible for the positive relationships between diversity and the level and stability of ecosystem functioning include (1) species complementarity (species do different things; Cardinale *et al.*, 2007), (2) “sampling” effects (species-rich communities are more likely to include effective service providers (Duffy, 2009), (3) response diversity (species respond differently to changes in the environment; Elmqvist *et al.*, 2003; Gonzalez & Loreau, 2009), and (4) statistical averaging (ecosystem functioning is more stable in diverse communities because random fluctuations is averaged across species; Doak *et al.*, 1998).

Using ecosystem services for labelling the links between biodiversity and benefits for humans can be a powerful tool to inform and guide natural resource management and policy (Mace *et al.*, 2012). Different ecosystem services can be linked to each other via indirect interactions if they respond

similarly to changes in the environment, and via direct interactions if they directly affect each other (Bennett *et al.*, 2009). The ecosystem services pollination and pest control for example, can interact indirectly if management for one service has effect on the other (Shackelford *et al.*, 2013), and directly if the level of pest control affects pollination or vice versa. Interactions between ecosystem services have important practical implications for how to combine management of multiple services, but they remain poorly studied (Seppelt *et al.*, 2011).

### 3.2 Crop pollination

“Pollination is the process by which pollen is transferred in the reproduction of plants, thereby enabling fertilization and sexual reproduction”  
(definition of pollination according to Wikipedia 2013-03-01)

Pollination really seems to be a business for plants - so why is it then often of interest to insect ecologists? An important answer lies in the importance of insects as vectors for pollen. Almost 90 percent of the world's approximately 352 000 flowering plants species are at least partly pollinated by animals (Ollerton *et al.*, 2011) and some 99 percent of the 100 000-200 000 pollinator species are insects (Ingram *et al.*, 1996).

Turning the interest to crops, 87 of 115 leading world crops benefit from insect pollination, and insect pollination contributes with 9.5 percent of the total annual global value of crop production (Klein *et al.*, 2007; Gallai *et al.*, 2009). Further, as an example of the role of pollinators for nutrition, pollinator dependent crops contribute more than 90 percent of the vitamin C that humans consume (Eilers *et al.*, 2011).

While the exact contribution of *Apis* (honeybee) versus non-*Apis* pollinators for crop production remains unclear and debated (Allsopp *et al.*, 2008; Breeze *et al.*, 2011; Garibaldi *et al.*, 2013), the managed honey bee *Apis mellifera* L. is currently the single most important crop pollinator species. Over the last years, significant problems with honey bee health, including Colony Collapse Disorder, has been observed in honeybees (Neumann & Carreck, 2010). These bee health problems might have arisen from a not yet fully understood combination of factors, including increased pest and pathogen pressures, malnutrition, agrochemicals, apicultural mismanagement and lack of honey bee genetic diversity (Potts *et al.*, 2010). As the agricultural demand for pollination has already for several decades been outgrowing the global supply of honey bee colonies (Aizen & Harder, 2009), bee health problems further

limiting the growth of the global honey bee stock might pose a threat to food security.

Wild bees is the dominant or even essential group of unmanaged pollinators providing crop pollination services for a number of crop systems (Klein *et al.*, 2007; Winfree *et al.*, 2007; Garibaldi *et al.*, 2013). There is evidence that wild bees, especially bumble bees, are declining (Biesmeijer *et al.*, 2006; Williams *et al.* 2009; Dupont *et al.*, 2011; Bartomeus *et al.*, 2013). Habitat loss and fragmentation, that might have disassociated flowering and nesting resources in space and time, are considered to be the most important drivers of wild bee declines (Winfree *et al.*, 2009; Williams *et al.*, 2012; Kennedy *et al.*, 2013), but agrochemicals (Brittain & Potts, 2011; Rundlöf *et al.*, 2012), and pathogens (Cameron *et al.*, 2011; Murray *et al.*, 2013) may also play a role. Wild pollinator declines can have negative consequences for crop pollination (Garibaldi *et al.*, 2011a; 2011b), but more studies would be needed in order to determine whether a ‘pollination crisis’ is imminent or not (Ghazoul, 2005; Steffen-Dewenter *et al.*, 2005)

### 3.3 Pest control

In a global perspective, animal pests, primarily insects, typically reduce yields in all major crops by 5 to 15 percent despite control efforts (Oerke *et al.*, 1994; Oerke & Dehne, 2004). Huge resources have been invested in crop protection during the 20th century, primarily as chemical control. Despite this, crop yield losses to pests, pathogens and weeds have increased in the period from 1965 to 1990, both in absolute and proportional terms (Oerke *et al.*, 1994). An important explanation is that the intensification measures that have increased yields, for example increased nitrogen input, cultivar changes, and denser monoculture crop stands, have also been very favourable for crop pests (Oerke & Dehne, 1997). Yet another possible explanation for increased crop losses to pests is that pesticide use has deteriorated naturally occurring pest control through negative impacts on non-target natural enemies in many cropping systems (Ekström & Ekbom, 2011).

The concept of integrated pest management (IPM) to achieve sustainable pest control was established as a reaction to the pesticide backlash in the 1960s, when the negative side effects of pesticides for human health, non-target organisms and the environment became evident and received public attention (Stern *et al.*, 1959; Carson, 1962; Metcalf, 1980). Although IPM comes with many definitions, it usually implies the use of a number of suitable techniques in a compatible manner to keep pests below the level at which they cause economic damage, using chemicals only as a backup option (Kogan,



1998; Zalucki *et al.*, 2009). New generations of pesticides with lower acute toxicity have been developed, but negative effects of pesticide use on biodiversity, the environment and humans remain (Devine & Furlong 2007; Geiger *et al.*, 2010; Rundlöf *et al.*, 2012). Given the scientific and policy interest in IPM, more IPM and alternatives to chemical control strategies would be expected in agricultural practice. However, global expenditures on pesticides have continued to increase steadily (FAO, 2012). Research gaps on how different pests affect crop yield, at what level of pest attack pesticide use is economically beneficial, and on alternative control options have been identified as major obstacles for the implementation of IPM (Wearing, 1988; Zalom, 1993; Zalucki *et al.*, 2009).

While it is evident that yield losses from insect pest attacks generally would be higher without the presence of natural enemies that occur naturally in agricultural fields, a general estimate of the contribution of biological control to pest suppression and yield enhancement is often lacking. This might be explained by difficulties in estimating and up-scaling economic valuations of biological pest control services. However, in a rare such up-scaled study, Losey & Vaughan (2006) provided the conservative estimate that natural enemies contributed with about 4 percent of the annual production value of crops in the U.S.

Studies on biological control on aphids, one of the most damaging groups of invertebrate pests world-wide, provide a valuable exception where more is known about the value of biological control. Because of the short generation time in aphids, the effect of predator exclusion on these pests can be followed over multiple generations within a short time period. A recent meta-analysis reviewed 168 individual cases where natural enemies to aphids were excluded from different plants and found that this increased aphid abundances in 132 (79%) of the cases (Diehl *et al.*, 2013). Effects of natural enemies on aphids can then be related to the aphids' effects on the crop, allowing for a calculation of the contribution of natural enemies to crop yields. Such studies show significant yield increases attributable to natural enemies, especially in cropping systems not relying on pesticides for pest control (Östman *et al.*, 2003; Landis *et al.*, 2008). For example, in a Swedish case study by Östman and colleagues (2003), 23 percent of the barley yield was attributable to generalist natural enemy suppression of aphid damage.

Our knowledge is limited on the long term persistence of natural enemy populations in modern agricultural landscapes which have little natural habitat left and where the use of pesticides is frequent. Studies repeatedly suggest, however, that natural enemy populations can be disrupted by insecticide use, and that decreased natural enemy exposure to insecticides will benefit

biocontrol services (Metcalf *et al.*, 1980; Settle *et al.*, 1996; Lu *et al.*, 2012). Moreover, complex landscapes with more non-crop habitats that offer natural enemies alternative hosts, nectar and pollen plants and hibernation sites, often harbour more numerous and species rich assemblages of natural enemies (Bianchi *et al.*, 2006; Chaplin-Kramer *et al.*, 2011). Specialised and poorly dispersing natural enemies may be particularly favoured in such landscapes (Tscharntke *et al.*, 2007). It remains an unresolved question, however, whether the second trophic level, the herbivorous pests, generally are more or less abundant in complex agricultural landscapes (Chaplin-Kramer *et al.*, 2011).

### 3.4 Red clover and seed production of red clover

“*To be in clover*” - live luxuriously (clover being extremely delicious and fattening to cattle). (Online etymology dictionary)

Clovers (*Trifolium* spp.), a genus of plants native for Europe and the Middle East, are relative latecomers in agriculture. Clover was probably domesticated in Moorish Spain early in the last millennium and spread northwards in Europe from there (Kjargaard, 2003). It was introduced as a crop in Sweden in the mid 1700s (Kåhre, 1996). The nitrogen fixation ability of clover in times of severely nitrogen limited yields, and the value as a fodder crop soon made clover a success story. In fact, leguminous crops dominated by clover have been pointed out as the single most important factor for the near doubling of yields in Europe between 1770 and 1880 (Chorley, 1981). Fields of legumes largely replaced what earlier had been fallow land during this period, and it has been estimated that legumes were grown on approximately 19 percent of all agricultural land in Northern Europe in 1880, 7 percent of which were clover monocultures (Chorley, 1981).

Red clover is now grown around the globe, with certain regions and countries specialising on seed production, such as the southern-central parts of Sweden (Taylor & Quesenberry, 1996). The invention of the Haber-Bosch method to produce nitrogen fertilizers, coupled with plentiful amounts of cheap fossil fuel available to perform this energy demanding process, meant that clover lost its importance gradually over the twentieth century (Taylor & Quesenberry, 1996). The acreage for seed production of red clover consequently decreased with about 90% in Sweden 1939-2010 (Witte, 1940; SFO 2013). However, with an end of the era of cheap fossil energy possibly approaching, the interest in alternatives to inorganic fertilisers is increasing and clover might again increase in importance (Kjargaard, 2003).

Red clover grown for seed in Sweden is normally sown in together with a spring cereal crop in the first year, harvested in the second year and then the field is rotated. Both diploid and tetraploid cultivars are grown, the latter producing more forage but also tending to yield less seeds. Flowering time is June to August in the harvesting year. Seeds are harvested late in summer or early in autumn some 1.5 to 2 months after full bloom. Sweden is among the largest producers of red clover seed in Europe with a current acreage a little over 2'000 ha. Seed prices are high (ca. 20 to 50 SEK per kg), and variation in yield is huge (0 to ca. 800 kg/ha), making red clover seed a risky cash crop. The causes of the yield variation are not fully known, but cultivar, establishment, pollination, pest control, harvesting method, and weather conditions, especially around harvesting time, may all play a role. Apart from yield quantity, seed germination rates and weed seed content are also important yield parameters. Red clover for seed tends to be grown by experienced and specialised farmers (Jonsson, 2011).

#### 3.4.1 Red clover pollination

“I have also found that the visits of bees are necessary for the fertilisation of some kinds of clover; for instance twenty heads of Dutch clover (*Trifolium repens*) yielded 2,290 seeds, but twenty other heads, protected from bees, produced not one. Again 100 heads of red clover (*T. pratense*) produced 2,700 seeds, but the same number of protected heads produced not a single seed.”  
(Darwin, 1859)

Red clover is obligately out-crossed which means that insect pollinators are essential for seed production. A red clover plant typically flowers for several weeks, and an inflorescence (flower-head) normally has 50-200 flowers which open continuously over 6-10 days. Successful pollination normally leads to the development of a single seed per flower (Free, 1993). Bumble bees (*Bombus* spp.) and honey bees (*Apis mellifera* L.) are the dominating pollinators of red clover, although occasionally other bee species are also reported as pollinators (Free, 1993; Rao & Stephen, 2009; Fig. 1).

Because not all bees can easily reach the nectar in red clover corollas, bees with longer tongues will work faster and visit more red clover flowers per time period (Nørgaard Holm, 1966). This implies that bumble bees will pollinate more flowers per time unit compared to honey bees, and that bumble bee species with longer tongue lengths will pollinate the most per time unit. Compared to honey bees, bumble bees start visiting red clover flowers earlier

in the morning and keep on visiting later in the evening (Schwan, 1953). They also arrive to red clover fields earlier in bloom and stay longer towards the end of the blooming period. However, under suitable conditions of warm and sunny weather in mid-bloom, honey bees can greatly outnumber bumble bees as pollinators in red clover by sheer numbers (Schwan, 1953).

Nectar robbing (Irwin *et al.*, 2010) complicates the picture of how much different bee species contribute to pollination of red clover. *Bombus terrestris* L. and *Bombus lucorum* L. sometimes bite a hole at the bottom of the corolla to obtain nectar, without contacting the sexual parts of the plant (“primary robbers”, Nørgaard Holm, 1966). Other bumble bee species as well as honey bees can subsequently obtain nectar from the bite-holes of primary robbers (“secondary robbers”, Free, 1993). There is considerable variation in the relation between positive visits of pollen and nectar collecting bees and negative visits of primary and secondary robbing performed by the pollinator community of red clover. Factors that will positively affect the proportion of positive visits include (1) low numbers of primary robbing bumble bee species, (2) high nectar levels in the plants and (3) short corolla tube lengths of the flowers (Schwan, 1953).

#### 3.4.2 Red clover pest control

“Having ordered a field of clover, consisting about eight acres, to be saved for seed, my servant, on the 9th of August last, asked me whether I chose to save the whole field for that purpose, saying that he had examined several of the heads, and found the maggot in them.” (Markwick, 1802)

A diversity of weevil species in the genres *Apion*, *Hypera* and *Sitona* are known to feed on red clover (Schnell, 1955). *Hypera* spp. weevils have been reported as important pests in white clover grown for seed production in Denmark (Langer & Rohde, 2005; Hansen & Boelt, 2008). Occasionally *Sitona* spp. weevils are reported to damage newly emerged red clover seedlings in Sweden (Andersson *et al.*, 2012a). The major pest insects in European red clover seed production is, however, *Apion* spp. weevils (Coleoptera: Brentidae), and in particular the clover seed weevils *A. trifolii* L., *A. apricans* Hbst. and *A. assimile* Kirby (Notini, 1935; Markkula *et al.*, 1964).

The clover seed weevils hibernate as adults under shelter in dry and protected habitats outside the fields such as forest edges, under juniper or in tussocky grass (Jones, 1950; Ohlsson, 1968). They infest clover fields in the spring both by crawling and, at higher temperatures, also by flying into the

field (Ohlsson, 1968). Adult *Apion* spp. weevils feed on clover leaves without causing any severe damage to the plant (Jones, 1950). The adult weevils oviposit in clover inflorescence buds and the larva develops inside the inflorescence, where it consumes 6-10 ovules and developing seeds (Jones, 1950; Fig. 1). Laid eggs hatch after 8-10 days, the larvae develop through three instars in 16-26 days and hatch as adults after 8-9 days of pupation (Bovien & Jørgensen, 1934; Notini, 1935). The hatched juveniles remain and feed in the field for some time and then migrate back to overwintering habitats in the autumn (Schnell, 1955).

Parasitic wasps within the order Hymenoptera attack the larvae of clover seed weevils, which can result in high parasitisation rates (Kruess & Tscharnkte, 1994; Kruess, 1996; Fig. 1). The biological information available on the parasitoids is only fragmentary. The most common species *Spintherus dubius* Nees is a solitary idiobiont parasitoid which lays its egg under the cuticle of medium-to-late instars of weevil larva (Notini, 1935; Kruess, 1996). In Britain, two generations of imagines of this species appear per year (in April to May and in August to September), and like related Pteromalid species also parasitizing clover seed weevils, females probably overwinter as adults in dry locations (Graham, 1969). Biological control remains largely unexplored in commercial clover seed production.

Pest control of clover seed weevils in Sweden is based on insecticide use with limited knowledge on pest sampling methods to determine pest infestation rates or yield losses, and with no economic threshold which specifies the pest infestation rate at which control methods are economically beneficial (Andersson *et al.*, 2012a). There is some support that maximising the distance between white clover fields from one season to the next can limit pest abundance (Langer & Rohde, 2005), but this remains to be evaluated in red clover seed production.



*Figure 1.* Study organisms: a bumble bee pollinating a red clover flower (top left), an adult clover seed weevil on a budding red clover plant (bottom right), two weevil larva developing inside a withered inflorescence (flower-head cut in half; bottom left), and a hymenopteran parasitoid reared from a red clover sample (top right). Photos by Linnea Bergström (bee), Vita Manak (parasitoid) and Ola Lundin (weevils).

## 4 Methods

Trends for functionally important insects were analysed by comparing data collected in the field with historical data collected from the literature (paper I,III). Yield impacts were examined historically over time by compiling yield data from the literature (paper I), it was examined in field studies (paper II), and it was examined in a field experiment (paper IV). Management options for pest control was explored in field studies by excluding plots from insecticide treatments (paper II), and by analysing the impact of natural variation in management, landscape and climate factors on pest control (paper III).

Field sites in paper I were distributed throughout Sweden, while field work for other studies were conducted in the region of Skåne in southernmost Sweden. Field sites were commercially grown red clover seed fields which were selected to overlap historically sampled sites (paper I, III), to capture maximum variation in landscape composition and climatic conditions (paper II-III), or to represent contrasting levels of pest control (paper IV). Farmers were contacted with assistance from seed companies in the spring of each field season for permission to work in their fields. Participating farmers were regularly updated with data from their field and project results 2008-2011.

General or Generalized Linear Mixed Models were typically used to statistically evaluate data. Mixed models have the useful property that they can contain both fixed explanatory variables and random effects. Non-independence in data can be accounted for by specifying random effect structures in mixed models, and statistical conclusions from mixed models can be generalised to the population level for random effects (Quinn & Keogh, 2002). Common examples of random factors in biology are for example sites and plots. The difference between general and generalized models is that the latter is a more general framework that does not assume normally distributed errors (Bolker *et al.*, 2009).

## 4.1 Temporal trends for crop pollinators, pests and natural enemies

We scanned for historical data on crop pollinators, pests, and natural enemies in all known publications from the Swedish association for seed growing farmers (*Frö- och oljeväxtodlarna*) as well as in national crop protection bulletins. Additionally, data on the relative abundance over time of the two dominating seed weevil species *A. apricans* and *A. trifolii* was retrieved from the Swedish Reporting System for Terrestrial and Limnic Evertebrates ([www.artportalen.se](http://www.artportalen.se)).

Field data on bumble bee community composition was collected from 83 red clover seed fields over three years on locations across Sweden that largely matched historically sampled sites. Flower visiting bumble bees were collected with sweep-nets along 1 m wide and 50 m long transects (fig. 2). Each site was sampled two to five times on days with warm, sunny and calm weather during crop bloom, typically both at the edge and in the centre of the fields. Collected bumble bees were determined to species in the laboratory following Løken (1973), Prys-Jones & Corbet (1987), and Edwards & Jenner (2005).

We reared clover seed weevils and their parasitoids from a total of 53 fields in southernmost Sweden over 4 years on locations that largely matched historically sampled sites. Juvenile seed weevils and natural enemy parasitoids emerging from inflorescences were sampled by rearing them following established protocols (Notini, 1935; 1938; Markkula *et al.*, 1964). Inflorescences were collected from each field and put in cardboard boxes with a hole on the side. A transparent tube was put in the hole to collect emerging weevils and parasitoids (fig. 2). Based on earlier reports of red clover seed weevil parasitoids (Notini, 1935; Kruess, 1996), two emerging morphospecies of wasps, Pteromalidae and Helconinae, were identified as parasitoids of clover seed weevils and therefore considered in further analyses of clover seed weevil parasitism. A subsample of wasps within these morphospecies was further determined to species with the help from taxonomic experts in order to confirm the taxonomic identities and make comparisons to earlier studies.

## 4.2 The role of insects for crop yield

We compiled data on seed yields in red clover in Sweden from various publications from the Swedish association for seed growing farmers (*Frö- och oljeväxtodlarna*) and analysed trends in the level and stability of yields.

Seed yield was measured on most of the fields where insect data was collected. Several one square meter plots were manually harvested with hand clippers in each field (fig. 2). Harvesting samples were dried, threshed, rinsed





*Figure 2.* Some of the methods used for field data collection: Bumble bee sampling (top left), pan trapping of weevils (bottom left), rearing boxes for weevils and parasitoids (top right) and experimental harvesting of clover seeds (bottom right). Photos by Maj Rundlöf (harvesting) and Ola Lundin (other).

and weighed on an experimental farm operated by the Swedish Rural Economy and Agricultural Society (*Hushållningssällskapet*). Such a measure of yield is likely to be slightly higher, but still be related to, the actual seed yield harvested by the farmer. The impact of pest abundance on seed yield was examined in paper II.

In paper IV we manipulated pollination and pest control in an experiment and measured the effect on seed yield. The basic idea was to establish two contrasting levels of pest control on different plants, and then to perform a pollination experiment on the plants with two orthogonal levels of pollination. Six different fields were located that either had low or a high level of clover seed weevil abundances. We utilised the replicated contrasts in pest abundances as a simulation of two levels of pest control. Experimental clover plants were placed at the sites in the bud stage of the crop. Pollination was experimentally manipulated by relocating the experimental clover plants to cages with bumble bees (*Bombus terrestris* L.) and pollinating them for contrasting time periods (fig. 3). Shorter time exposure to bumble bees resulted in ‘low’ pollination, and longer time in ‘high’ pollination. The decision on which time spans would represent ‘low’ or ‘high’ pollination levels were based on pilot observations in the field. In this experiment we used seed set as a measure of yield. We verified that seed set was likely to be a good proxy for yield (paper IV, data supplement). To measure seed set, we manually inspected the calyx of all individual flowers on 10 flower-heads from each plant in the experiment. The proportion of calyces with a whole seed, the proportion of weevil damaged ovules (indicated by the calyx having a bite hole and not containing a whole seed), and the proportion of empty undamaged calyces was determined for each inflorescence inspected.

### 4.3 Management options for pest control

We tested a sampling method to forecast pest damage early in the season (paper II). Adult weevils can be sampled in clover with pan traps at ground level in the crop (Folkesson, 2005), by sweep-netting (Ohlsson, 1968), with D-vac (Topbjerg & Ytting, 2009), or with a collection net front-mounted on a motorcycle (Ellsbury & Davis, 1982). We chose to use pan traps (fig. 2), firstly because this method should be less sensitive to weather conditions or operator skills and secondly because it is a method which should be fairly easy for advisers and farmers to use. The pan traps are probably primarily catching weevils passively as they are crawling around in and fall down from the vegetation above the trap. Caught *Apion* weevils were determined to species

following Bovien & Jørgensen (1934), Notini (1935), and Gønget (1997). Catches in pan traps were then compared to the number of seed weevils hatched per inflorescence.

We evaluated chemical control options by excluding field zones from insecticide treatments (paper II). Data was collected on pest abundances, parasitism rates provided by natural enemies and on seed yields in untreated as well as treated field zones with previously described methods. Additional data on field and farm management was collected from participating farmers. More specifically, we received detailed records of insecticide use for each field which we used in paper II, and data on the location or estimated shortest distance to a red clover field in the previous season which we used in paper III. Landscape composition data used in paper III was calculated from spatially explicit data on agricultural land-use from the Integrated Administration and Control System (IACS) provided by the Swedish Board of Agriculture and interpreted in ArcGIS. Weather data used in paper III was received from the nearest weather station run by the Swedish Meteorological and Hydrological Institute (SMHI).



*Figure 3.* Cages used for pollination experiment. Photo: Linnea Bergström.



## 5 Results and discussion

### 5.1 Temporal trends for crop pollinators, pests and natural enemies

We found that there has been a historical shift in the composition of bumble bees in Swedish red clover fields (paper I). The relative abundances of the two short tongued bumble bees *B. terrestris* and *B. lapidarius* have increased from 40 percent in the 1940s to 89 percent in present communities, whereas several more long-tongued species had declined in relative abundance (Fig. 4). Such communities with a few, less efficient pollinator species dominating, is expected to deliver lower and less stable levels of pollination to red clover. Parallel historical trends of land use conversion, with spatial separation of agricultural land from semi-natural habitat, and decreased cropping of legumes might have contributed to the observed shift in pollinator composition. As long-tongued bumble bee species generally are more diet specialised on flowers with deeper corollas (Goulson *et al.*, 2005), this might explain why long tongued species has declined relative to short tongued bumble bee species in landscapes which now offers fewer such flowering resources (Carvell *et al.*, 2006; Kleijn & Raemakers, 2008). A Danish study on similar data suggested that short tongued bumble bee species were equally or more abundant in red clover fields now compared to in the 1930s, whereas long tongued had become rarer, or even gone extinct (Dupont *et al.*, 2011)

In paper III we found that abundances of clover seed weevils had more than doubled compared to pest abundances recorded in a historic dataset collected before the onset of pesticide use in the 1930s (Fig. 5). We furthermore found that there has been a shift in the species composition of red clover seed weevils in the study area. *A. trifolii*, which historically has had a limited southern distribution in Northern Europe, now dominated records. A recent increase of

this species is probably an important explanation for the increased pest abundances today. Furthermore, data indicated that historic parasitism rates were higher compared to current rates of clover seed weevil parasitism. It seems that the host-parasitoid interaction has tilted in favour of the pests, but further studies would be needed to determine if lower parasitism rates are a cause or effect of higher pest abundances.

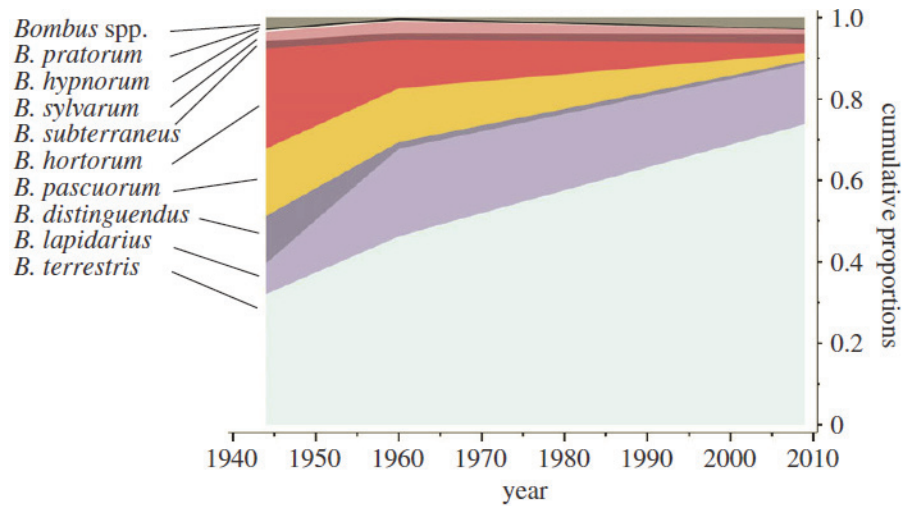


Figure 4. Proportional shifts in bumble bee community composition in the last 70 years. Proportion of bumble bee abundance for the different species is presented as cumulative proportions for the communities.

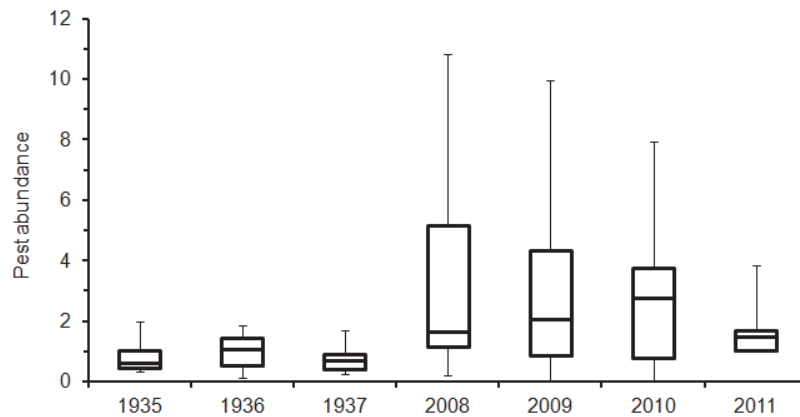


Figure 5. Boxplot showing historic and present pest abundances (Apion weevils per inflorescence) in red clover fields. Boxes show the 25<sup>th</sup> percentile, median and the 75<sup>th</sup> percentile values, while whiskers show minimum and maximum values.

## 5.2 The role of insects for crop yield

Historical yield data showed that seed yields have declined in recent decades and that the inter-annual variability in yields has increased (paper I, Fig. 6). Shifted levels of pollination and pest control most likely have contributed to this, but it is not possible to infer causal relationships from these data. However, in a more detailed analysis we could quantify a strong negative effect of clover seed weevils on seed yield, with mean yield losses of 30 to 35 percent in non-sprayed or pyrethroid-only sprayed crops (paper II, Fig. 7). Further studies are needed, that in more detail explore the importance of pollination for crop yield in the field. More specifically, it would be interesting to know how commonly and under what conditions yield is limited by pollination.

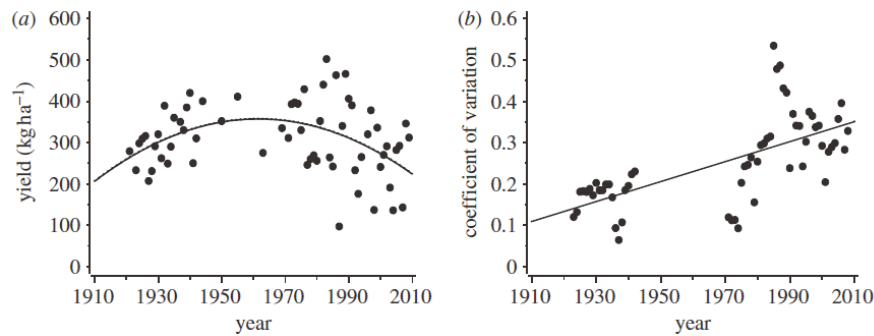


Figure 6. Trends in red clover seed yields in the last 90 years. (a) Yearly yield statistic and (b) variability in yield (CV, 5 year moving average).

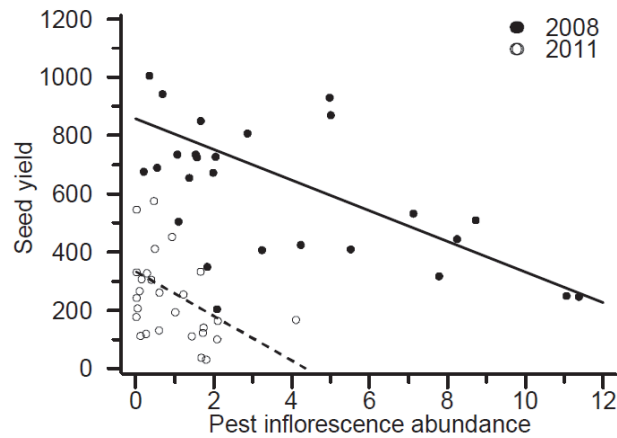


Figure 7. Seed yield (kg/ha) in relation to pest abundance (Apion weevils per inflorescence) in 2008 and 2011.



In the experimental cage study, the level of pollination and pest control individually had positive effects on red clover seed set (paper IV). There was, however, also a synergistic interaction; the gain in seed set obtained when simultaneously increasing pollination and pest control outweighed the sum of seed set gains obtained when increasing each of them in separation (Fig. 8). Reduced pollinator visitation to damaged plants or functional response of the pests to increased resource levels (more developing seeds) at high pollination levels might have contributed to this result. Despite its applied importance for crops, interactions between pollinators, pests and their control agents has so far mostly only be investigated from a plant evolutionary and demographic perspective (Strauss & Irwin, 2004; but see Strauss & Murch, 2004; Barber *et al.*, 2012), and therefore it deserves further study in cropping systems.

In summary, these results suggest strong effects of both pollination and pest control on yield in our model system, and that interactions can alter the benefits or damage received from functionally important insects. This needs to be better understood and considered to properly manage multiple ecosystem services.

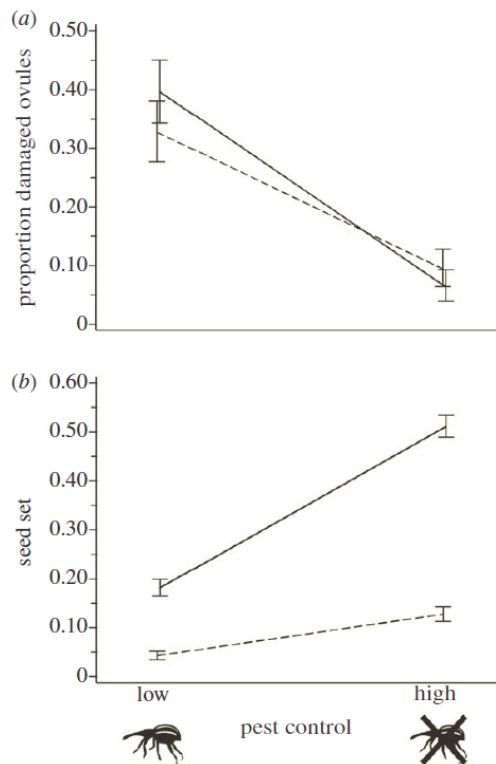


Figure 8. Mean proportion of damaged ovules (a) and mean proportion seed set (b) in relation two experimental pollination and pest control levels in red clover. Error bars show one standard error. Solid line, high pollination; dashed line, low pollination.



### 5.3 Management options for pest control

We found that pest damage could be predicted with pan traps (paper II). Pyrethroids was an ineffective chemical control option for clover seed weevils, while the neonicotinoid compound thiacloprid provided pest control without negatively affecting parasitism rates. Based on these results we developed a threshold for chemical control and recommend thiacloprid to be applied when clover seed weevils abundances cannot be prevented from exceeding threshold levels. The safety of neonicotinoids for beneficial insects, especially bees, has been strongly questioned recently (Cresswell, 2011; Whitehorn *et al.*, 2012). These worrying results, however, exclusively consider other neonicotinoid compounds that are several orders of magnitude more toxic to bees and that degrade more slowly in plants and in the environment compared to thiacloprid (Iwasa *et al.*, 2004; Mommaerts *et al.*, 2010; Hopwood *et al.*, 2012). As a safety measure to reduce exposure to beneficial insects we, however, suggest thiacloprid to only be applied before the onset of flowering in red clover.

Abundances of clover seed weevils were higher in landscapes with a high proportion of agricultural land within 5 km of the field (paper III). Southernmost Sweden now also seems to be much more suitable for the clover seed weevil *A. trifolii* in comparison to historic times. There is a strong tradition of growing red clover for seed in southern Sweden, but it might be that climate and landscape changes have resulted in that optimal growing conditions have shifted northward in the country. Similar to earlier results in white clover (Langer & Rohde, 2005), the presence of a source population (i.e. a red clover seed field in the previous season) within 800 meters also tended to increase the abundances of clover seed weevils. A management option is therefore to as far as possible avoid red clover seed fields close to each other in subsequent years. More research to advance integrated pest management in this crop is needed. Clover seed weevils probably use olfactory cues to locate their host plant (Andersson *et al.*, 2012b), and one interesting line of work is to explore if this can be exploited for pest control. More ecological knowledge of the main parasitoid species is also needed in order to develop conservation biological control options (Barbosa, 1998; Jonsson *et al.*, 2008).

A general challenge for the implementation of IPM is that the decision of whether to use a pesticide for control often involves an individual farmer weighing the short term risk of crop loss against long term benefit of resource conservation for society as well as the farmer (for example long term pest control and less pollution, Brewer & Goodell, 2012). This is a fundamental problem for IPM which might be solved by economically integrating societal benefits into pest management decisions.



## 6 Conclusions and future perspectives

Our results suggest that continued agricultural intensification with structural simplification of landscapes and increased use of external inputs jeopardises pollination, pest control and ultimately crop yields. Findings supporting this were based on data compiled from the annals of applied research, which was compared with data collected in the field 2008-2011. More can probably be learned about the trends for crop pollinators, pests and natural enemies by repeating this for other crops and countries. There is, however, at some point a trade-off between allocating efforts to either learn more on the trends and their underlying drivers, or redirect resources to manage biodiversity trends in a preferred direction (Grantham *et al.*, 2009).

Agricultural land will likely be increasingly needed for the production of food and other products, which means that careful decisions must be made before land is taken out of direct production. Improving the integration of management options for biodiversity and ecosystem services in agricultural landscapes is probably going to be needed. Conservation efforts that benefit multiple taxa and functions will likely be more cost-effective. We showed that pollination and pest control might have interactive effects on crop yield. Plants depend on several services (e.g. pathogen control) and inputs (e.g. fertilisers) that might have interactive effects on plant performance and crop yields. The separate and joint (interactive) effects of these need to be better understood.

Ecological intensification entails enhancement of crop productivity by including regulating and supporting ecosystem services management in agricultural practices (Bommarco *et al.*, 2013). Ecological intensification is a viable alternative to conventional intensification measures in agriculture and might be able to meet future demand for food and other products while reducing environmental externalities and the use of off-farm inputs. For pest control, which was specifically targeted in this thesis, ecological intensification in the form of assisting pest control by natural enemies probably needs to be complemented by a range of other pest control methods. Proactive methods

like a planned crop rotation, and breeding and growing healthy and resistant crops should be prioritised, but sustainable and environmental friendly reactive inputs which can protect crops from pest attacks which cannot be prevented by other methods are probably also going to be needed (Zehnder *et al.*, 2007; Flint, 2012).

The way biodiversity conservation should be practised is likely dependent on whether the focus is species conservation, or conservation of species which provide ecosystem services (Kleijn *et al.*, 2011). Many species are dependent on large intact natural habitats to sustain their populations. For conservation of such species, it has been argued that limiting the expansion of agricultural areas and instead more efficiently use the agricultural land we have (“land-sparing”) is a better management option (Phalan *et al.*, 2011). For components of biodiversity that might be important to conserve or manage in agricultural landscapes because they can impact agricultural productivity “land-sharing”, i.e. the integration of agricultural production and conservation of biodiversity that supports productivity might be a better management strategy (Tscharntke *et al.*, 2012b).

Not to forget, significant steps towards sustainable global food production can be taken on the consumer and post-harvest side of the global food production equation. 30 to 40 percent of all food produced is currently lost due to waste, mainly in retail and by the end consumer in the developed world and mainly in the delivery chain in the developing world (Godfray *et al.*, 2010). There is also scope to support more protein production without increasing the area or intensity of crop production if animal protein is swapped for vegetable protein sources in the human diet (Godfray *et al.*, 2010). Finally, there is a large scope for more equal per capita distribution of the food produced globally (Gardner & Halveil, 2000).

## References

- Aizen, M.A. & Harder, L.D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Current Biology*, 19, 915-918.
- Allsopp, M.H., de Lange, W.J. & Veldtman, R. (2008). Valuing insect pollination services with cost of replacement. *PLoS ONE*, 3, e3128.
- Andersson, G., Arvidsson, A., Berg, G., Djurberg, A., Gustafsson, G., Holmblad, J., Johansson, L., Karlsson, A., Lerenius, C., Sandström, M. & Wikström, M. (2012a). *Bekämpningsrekommendationer svampar och insekter 2012*. Swedish Board of Agriculture, Jönköping.
- Andersson, M.N., Larsson, M., Svensson, G.P., Birgersson, G., Rundlöf, M., Lundin, O., Lankinen, Å. & Anderbrant, O. (2012b). Characterization of olfactory sensory neurons in the white clover seed weevil, *Apion fulvipes* (Coleoptera: Apionidae). *Journal of Insect Physiology*, 58, 1325-1333.
- Barber, N.A., Adler, L.S., Theis, N., Hazzard, R.V. & Kiers, E.T. (2012). Herbivory reduces plant interactions with above-and belowground antagonists and mutualists. *Ecology*, 93, 1560-1570.
- Barbosa, P. (1998). *Conservation biological control*. Academic Press, San Diego, CA, USA.
- Bartomeus, I., Ascher, J.S., Gibbs, J., Danforth, B.N., Wagner, D.L., Hedtke, S.M. & Winfree, R. (2013). Historical changes in northeastern US bee pollinators related to shared ecological traits. *Proceedings of the National Academy of Sciences of the USA*. In press.
- Bengtsson, J., Angelstam, P., Elmqvist, T., Emanuelsson, U., Forbes, C., Ihse, M., Moberg, F. & Nyström, M. (2003). Reserves, resilience and dynamic landscapes. *Ambio*, 32, 389-396.
- Bengtsson, J. (2010). Applied (meta)community ecology: diversity and ecosystem services at the intersection of local and regional processes. In: Verhoef, H.A. & Morin, P.J. (eds.). *Community Ecology*, pp. 115-130. Oxford University Press, New York, USA.
- Bennett, E.M., Peterson, G.D. & Gordon, L.J. (2009). Understanding relationships among multiple ecosystem services. *Ecology Letters*, 12, 1394-1404.
- Bianchi, F.J.J.A., Booij, C.J.H. & Tscharntke, T. (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B: Biological Sciences*, 273, 1715-1727.
- Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. (2006).

- Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313, 351-354.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.S.S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24, 127-135.
- Bommarco, R., Kleijn, D. & Potts, S.G. (2013). Ecological intensification: harnessing ecosystem services for food security. *Trends in Ecology & Evolution*, 28, 230-238.
- Bovien, P. & Jørgensen, M. (1934). Orienterende undersøgelser over angreb af snudebiller (Apion) i kløverhoveder. *Tidsskrift for Planteavl*, 40, 376-398.
- Breeze, T.D., Bailey, A.P., Balcombe, K.G. & Potts, S.G. (2011). Pollination services in the UK: how important are honeybees? *Agriculture, Ecosystems & Environment*, 142, 137-143.
- Brewer, M.J. & Goodell, P.B. (2012). Approaches and incentives to implement integrated pest management that addresses regional and environmental issues. *Annual Review of Entomology*, 57, 41-59.
- Brittain, C. & Potts, S.G. (2011). The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. *Basic and Applied Ecology*, 12, 321-331.
- Carvell, C., Roy, D.B., Smart, S.M., Pywell, R.F., Preston, C.D. & Goulson, D. (2006). Declines in forage availability for bumblebees at a national scale. *Biological Conservation*, 132, 481-489.
- Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F. & Griswold, T.L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences of the USA*, 108, 662-667.
- Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M. & Weis, J.J. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences of the USA*, 104, 18123-18128.
- Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.A., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S. & Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, 486, 59-67.
- Carson, R. (1962). *Silent spring*. Houghton Mifflin, Boston, USA.
- Chaplin-Kramer, R., O'Rourke, M.E., Blitzer, E.J. & Kremen, C. (2011). A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecology Letters*, 14, 922-932.
- Chorley, G.P.H. (1981). The agricultural revolution in Northern Europe, 1750-1880: Nitrogen, legumes, and crop productivity. *The Economic History Review*, 34, 71-93.
- Cresswell, J.E. (2011). A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology*, 20, 149-157.
- Darwin, C. (1859). *On the origins of species by means of natural selection*. Murray, London, UK.
- Devine, G.J. & Furlong, M.J. (2007). Insecticide use: contexts and ecological consequences. *Agriculture and Human Values*, 24, 281-306.
- Diehl, E., Sereda, E., Wolters, V. & Birkhofer, K. (2013). Effects of predator specialization, host plant and climate on biological control of aphids by natural enemies: a meta-analysis. *Journal of Applied Ecology*, 50, 262-270.

- Doak, D.F., Bigger, D., Harding, E.K., Marvier, M.A., O'Malley, R.E. & Thomson, D. (1998). The statistical inevitability of stability-diversity relationships in community ecology. *American Naturalist*, 151, 264-276.
- Dupont, Y.L., Damgaard, C. & Simonsen, V. (2011). Quantitative historical change in bumblebee (*Bombus* spp.) assemblages of red clover fields. *PLoS ONE*, 6, e25172.
- Duffy, J.M. (2009). Why biodiversity is important to the functioning of real-world ecosystems. *Frontiers in Ecology and the Environment*, 7, 437-444.
- Edwards, E. & Jenner, M. (2005). *Field guide to the bumblebees of Great Britain & Ireland*. Ocelli, Eastbourne, UK.
- Eilers, E.J., Kremen, C., Greenleaf, S.S., Garber, A.K. & Klein, A.-M. (2011). Contribution of pollinator-mediated crops to nutrients in the human food supply. *PLoS ONE*, 6, e21363.
- Ekström, G. & Ekbom, B. (2011). Pest control in agro-ecosystems: an ecological approach. *Critical Reviews in Plant Sciences*, 30, 74-94.
- Ellsberry, M.M. & Davis, F.M. (1982). Front-mounted motorcycle net for mass collection of clover insects. *Journal of Economic Entomology*, 75, 251-253.
- Elmqvist, T., Folke, C., Nyström, M., Peterson, G., Bengtsson, J., Walker, B. & Norberg, J. (2003). Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment*, 1, 488-494.
- FAO. (2012). *FAOSTAT pesticide import data*. Food and Agriculture Organization of the United Nations, Rome, Italy. <http://faostat.fao.org>.
- Fisher, B., Kerry Turner, R.K. & Morling, P. (2009). Defining and classifying ecosystem services for decision making. *Ecological Economics*, 68, 643-653.
- Flint, M.L. (2012). *IPM in practice: principles and methods of integrated pest management*. 2nd ed. University of California Agriculture and Natural Resources, Richmond, CA, USA.
- Folkesson, Ö. (2005). Klöverspetsvivar -förekomst och skada i klöverfröodlingar i södra Skåne. *Meddelanden från Södra Jordbruksförsöksdistriktet*, 58, 1-5.
- Free, J.B. (1993). *Insect pollination of crops*. Academic Press, London, UK.
- Gallai, N., Sallescu, J.M., Settele, J. & Vaissière, B.E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, 68, 810-821.
- Gamfeldt, L., Hillebrand, H. & Jonsson, P.R. (2008). Multiple functions increase the importance of biodiversity for overall ecosystem functioning. *Ecology*, 89, 1223-1231.
- Gardner, G. & Halweil, B. (2000). *Overfed and underfed: the global epidemic of malnutrition*. Worldwatch Institute, Paper 150, Washington, DC, USA.
- Garibaldi, L.A., Steffan-Dewenter, I., Kremen, C., Morales, J.M., Bommarco, R., Cunningham, S.A., Carvalheiro, L.G., Chacoff, N.P., Dudenhöffer, J.H., Greenleaf, S.S., Holzschuh, A., Isaacs, R., Krewenka, K., Mandelik, Y., Mayfield, M.M., Morandin, L.A., Potts, S.G., Ricketts, T.H., Szentgyörgyi, H., Viana, B., Westphal, C., Winfree, R. & Klein, A.M. (2011a). Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecology Letters*, 14, 1062-1072.
- Garibaldi, L.A., Aizen, M.A., Klein, A.M., Cunningham, S.A. & Harder, L.D. (2011b). Global growth and stability of agricultural yield decrease with pollinator dependence. *Proceedings of the National Academy of Sciences of the USA*, 108, 5909-5914.

- Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., Carvalheiro, L.G., Harder, L.D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N.P., Dudenhöffer, J.H., Freitas, B.M., Ghazoul, J., Greenleaf, S., Hipólito, J., Holzschuh, A., Howlett, B., Isaacs, R., Javorek, S.K., Kennedy, C.M., Krewenka, K., Krishnan, S., Mandelik, Y., Mayfield, M.M., Motzke, I., Munyuli, T., Nault, B.A., Otieno, M., Petersen, J., Pisanty, G., Potts, S.G., Rader, R., Ricketts, T.H., Rundlöf, M., Seymour, C.L., Schüepp, C., Szentgyörgyi, H., Taki, H., Tscharntke, T., Vergara, C.H., Viana, B.F., Wanger, T.C., Westphal, C., Williams, N. & Klein, A.M. (2013). Wild pollinators enhance fruit set of crops regardless of honey-bee abundance. *Science*, 339, 1608-1611.
- Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Pärt, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Oñate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hänke, S., Fischer, C., Goedhart, P.W. & Inchausti, P. (2010). Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology*, 11, 97-105.
- Ghazoul, J. (2005). Buzziness as usual? Questioning the global pollination crisis. *Trends in Ecology & Evolution*, 20, 367-373.
- Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. & Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *Science*, 327, 812-818.
- Gonzalez, A. & Loreau, M. (2009). The causes and consequences of compensatory dynamics in ecological communities. *Annual Review of Ecology, Evolution and Systematics*, 40, 393-414.
- Goulson, D., Hanley, M.E., Darvill, B., Ellis, J.S. & Knight, M.E. (2005). Causes of rarity in bumblebees. *Biological Conservation*, 122, 1-8.
- Graham, M.W.R.D.V. (1969). The Pteromalidae of North-Western Europe (Hymenoptera-Chalcidoidea). *Bulletin of the British Natural History Museum Entomology Supplement*, 16, 1-908.
- Grantham, H.S., Wilson, K.A., Moilanen, A., Rebelo, T. & Possingham, H. P. (2009). Delaying conservation actions for improved knowledge: how long should we wait? *Ecology Letters*, 12, 293-301.
- Gönnert, H. (1997). *The Brentidae (Coleoptera) of Northern Europe*. Brill Academic Publishers, Leiden, the Netherlands.
- Hansen, L.M. & Boelt, B. (2008). Thresholds of economic damage by clover seed weevil (*Apion fulvipes* Geoff.) and lesser clover leaf weevil (*Hypera nigrirostris* Fab.) on white clover (*Trifolium repens* L.) seed crops. *Grass and Forage Science*, 63, 433-437.
- Hanski, I. (1999). *Metapopulation ecology*. Oxford University Press, Oxford, UK.
- Hoekstra, J.M., Boucher, T.M., Ricketts, T.H. & Roberts, C. (2005). Confronting a biome crisis: global disparities of habitat loss and protection. *Ecology Letters*, 8, 23-29.
- Hopwood, J., Vaughan, M., Shepherd, M., Biddinger, D., Mader, E., Black, S. H. & Mazzacano, C. (2012). *Are neonicotinoids killing bees? A review of research into the effects of neonicotinoid insecticides on bees, with recommendations for action*. The Xerces Society for Invertebrate Conservation, Portland, USA.



- Ingram, M., Nabhan, G.P. & Buchmann, S.L. (1996). Our forgotten pollinators: protecting the birds and bees. *Global Pesticide Campaigner*, 6. PANNA, San Francisco, CA, USA.
- Ives, A. R. & Carpenter, S.R. (2007). Stability and diversity of ecosystems. *Science* 317, 58-62.
- Iwasa, T., Motoyama, N., Ambrose, J.T., & Roe, R.M. (2004). Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Protection*, 23, 371-378.
- Irwin, R.E., Bronstein, J.L., Manson, J.S. & Richardson, L. (2010). Nectar robbing: ecological and evolutionary perspectives. *Annual Review of Ecology, Evolution, and Systematics*, 41, 271-292.
- Jones, M. J. (1950). Clover seed weevils. *Annals of Applied Biology*, 37, 313-320.
- Jonsson, M., Wratten, S.D., Landis, D.A. & Gurr, G.M. (2008). Recent advances in conservation biological control of arthropods by arthropods. *Biological Control*, 45, 172-175.
- Jonsson, H. (2011). Masterclass i rödklöverfrö. *Svensk Frötidning*, 2, 7-10.
- Kennedy, C., Lonsdorf, E., Williams, N., Ricketts, T., Winfree, R., Bommarco, R., Brittain, C., Burley, A., Cariveau, D., Carvalheiro, L., Chacoff, N., Cunningham, S., Danforth, B., Dudenhöffer, J., Elle, E., Gaines, H., Gratton, C., Garibaldi, L., Holzschuh, A., Isaacs, R., Javorek, S., Jha, S., Klein, A., Krewenka, K., Mandelik, Y., Mayfield, M., Morandin, L., Neame, L., Otieno, M., Park, M., Potts, S., Rundlöf, M., Saez, A., Steffan-Dewenter, I., Taki, H., Wilson, J., Viana, B., Westphal, C., Greenleaf, S. & Kremen, C. (2013). A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecology Letters*. In press.
- Kjærsgaard, T. (2003). A plant that changed the world: the rise and fall of clover 1000-2000. *Landscape Research*, 28, 41-49.
- Kleijn, D. & Raemakers, I. (2008). A retrospective analysis of pollen host plant use by stable and declining bumble bee species. *Ecology*, 89, 1811-1823.
- Kleijn, D., Rundlöf, M., Scheper, J., Smith, H.G. & Tscharntke, T. (2011). Does conservation on farmland contribute to halting the biodiversity decline? *Trends in Ecology and Evolution*, 26, 474-481.
- Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274, 303-313.
- Kogan, M. (1998). Integrated pest management: historical perspectives and contemporary developments. *Annual Review of Entomology*, 43, 243-270.
- Kruess, A. & Tscharntke, T. (1994). Habitat fragmentation, species loss, and biological control. *Science*, 264, 1581-1584.
- Kruess, A. (1996). *Folgen der Lebensraum-Fragmentierung für Pflanze-Herbivor-Parasitoid-Gesellschaften: Artendiversität und Interaktionen*. Paul Haupt, Bern, Switzerland.
- Kåhre, L. (1996). *Från höfrö till vallfrö - den svenska fröförsörjningen 1740-1870*. Kungliga Skogs- och Lantbruksakademien, Stockholm, Sweden.
- Landis, D.A., Gardiner, M.M., van der Werf, W. & Swinton, S.M. (2008). Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. *Proceedings of the National Academy of Sciences of the USA*, 105, 20552-20557.

- Langer, V. & Rohde, B. (2005). Factors reducing yields of organic white clover seed production in Denmark. *Grass and Forage Science*, 60, 168-174.
- Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M. & Gonzalez, A. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters*, 7, 601-613.
- Levins, R. (1969). Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America*, 15, 237-240.
- Losey, J.E. & Vaughan, M. (2006). The economic value of ecological services provided by insects. *Bioscience*, 56, 311-323.
- Lu, Y., Wu, K., Jiang, Y., Guo, Y. & Desneux, N. (2012). Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature*, 487, 362-365.
- Løken, A. (1973). Studies on Scandinavian bumble bees (Hymenoptera, Apidae). *Norwegian Journal of Entomology*, 20, 1-218.
- Mace, G.M., Norris, K. & Fitter, A.H. (2012). Biodiversity and ecosystem services: a multilayered relationship. *Trends in Ecology & Evolution*, 27, 19-26.
- Markkula, M., Myllymaki, S. & Kanervo, V. (1964). The abundance of seed pests of red clover in Finland and the effect of certain factors on their abundance. *Annales Agriculturae Fenniae*, 3, 95-132.
- Markwick, W. (1802). Observations on the curculio trifolii, or clover weevil, a small insect which infests the heads of the cultivated clover, and destroys the seed. *Transactions of the Linnean Society of London*, 6, 142-146.
- McGill, B.J. (2010). Towards a unification of unified theories of biodiversity. *Ecology Letters*, 13, 627-642.
- Metcalf, L.R. (1980). Changing role of insecticides in crop protection. *Annual Review of Entomology*, 25, 219-256.
- MEA. (2005). *Millennium Ecosystem Assessment*. World Resources Institute, Washington D.C., USA.
- Mommaerts, V., Reynders, S., Boulet, J., Besard, L., Sterk, G. & Smagghe, G. (2010). Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology*, 19, 207-215.
- Murray, T.E., Coffey, M.F., Kehoe, E. & Horgan, F.G. (2013). Pathogen prevalence in commercially reared bumble bees and evidence of spillover in conspecific populations. *Biological Conservation*, 159, 269-276.
- Naeem, S., Bunker, D.E., Hector, A., Loreau, M. & Perrings, C. (eds.). (2009). *Biodiversity, ecosystem functioning and human wellbeing*. Oxford University Press, Oxford, UK.
- Neumann, P. & Carreck, N.L. (2010). Honey bee colony losses. *Journal of Apicultural Research*, 49, 1-6.
- Nørgaard Holm, S. (1966). The utilization and management of bumble bees for red clover and alfalfa seed production. *Annual Review of Entomology*, 11, 155-182.
- Notini, G. (1935). Undersökningar rörande på rödklöver levande spetsvivlar (Apion Herbst) 1. deras förekomst, levnadssätt och utvecklingshistoria. *Meddelande, Statens Växtskyddsanstalt*, 9, 1-63.

- Notini, G. (1938). Undersökning rörande på rödklöver levande spetsvivlar (Apion Herbst) 2. Ekologiska undersökningar. *Meddelande, Statens växtskyddsanstalt*, 22, 1-41.
- Oerke, E.C., Dehne, H.W., Schönbeck, F. & Weber, A. (1994). *Crop production and crop protection: estimated losses in major food and cash crops*. Elsevier Science Publishers, Amsterdam, Netherlands.
- Oerke, E.C. & Dehne, H.W. (1997). Global crop production and the efficacy of crop protection-current situation and future trends. *European Journal of Plant Pathology*, 103, 203-215.
- Oerke, E.C. & Dehne, H.W. (2004). Safeguarding production - losses in major crops and the role of crop protection. *Crop Protection*, 23, 275-285.
- Ohlsson, B. (1968). Studies of Apion species on clover in Sweden (Col., Curculionidae). I. Short range dispersal and choice of hibernation sites of species injurious to cultivated clover. *National Swedish Institute for Plant Protection Contributions*, 14, 97-121.
- Ollerton, J., Winfree, R. & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120, 321-326.
- Perrings, C., Naeem, S., Ahrestani, F., Bunker, D.E., Burkill, P., Canziani, G., Elmqvist, T., Ferrati, R., Fuhrman, J., Jaksic, F., Kawabata, Z., Kinzig, A., Mace, G.M., Milano, F., Mooney, H., Prieur-Richard, A-H., Tschirhart, J. & Weisser, W. (2011). Ecosystem services, targets, and indicators for the conservation and sustainable use of biodiversity. *Frontiers in Ecology and the Environment*, 9, 512-520.
- Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25, 345-353.
- Phalan, B., Onial, M., Balmford, A. & Green, R.E. (2011). Reconciling food production and biodiversity conservation: land sharing and land sparing compared. *Science*, 333, 1289-1291.
- Prÿs-Jones, O.E. & Corbet, S.A. (1987). *Bumblebees*. Cambridge University Press, Cambridge, UK.
- Quinn, G.P. & Keough, M.J. (2002). *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge, UK.
- Rao, S. & Stephen, W.P. (2009). Bumble bee pollinators in red clover seed production. *Crop Science*, 49, 2207-2214.
- Robinson, R.A. & Sutherland, W.J. (2002). Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*, 39, 157-176.
- Rundlöf, M., Lundin, O. & Bommarco, R. (2012). *Växtskyddsmedlens påverkan på biologisk mångfald i jordbrukslandskapet*. KompetensCentrum för Kemiska Bekämpningsmedel, SLU, Uppsala.
- Schnell, W. (1955). Synökologische Untersuchungen über Rüsselkäfer der Leguminosekulturen. *Zeitschrift für angewandte Entomologie*, 37, 192-238.
- Schwan, B. (1953). Iakttagelser rörande rödklöverpollinerande insekter åren 1942-1946. *Meddelande från Sveriges Fröodlareförbund*, 2, 34-61.
- Seppelt, R., Dormann, C.F., Eppink, F.V., Lautenbach, S. & Schmidt, S. (2011). A quantitative review of ecosystem service studies: approaches, shortcomings and the road ahead. *Journal of Applied Ecology*, 48, 630-636.

- Settle, W.H., Ariawan, H., Astuti, E.T., Cahyana, W., Hakim, A.L., Hindayana, D., Lestari, A.S. & Pajarningsih, S. (1996). Managing tropical rice pests through conservation of natural enemies and alternative prey. *Ecology*, 77, 1975-1988.
- Shackelford, G., Steward, P.R., Benton, T.G., Kunin, W.E., Potts, S.G., Biesmeijer, J.C. & Sait, S.M. (2013). Comparison of pollinators and natural enemies: a meta-analysis of landscape and local effects on abundance and richness in crops. *Biological Reviews*. In press.
- Steffan-Dewenter, I., Potts, S.G. & Packer, L. (2005). Pollinator diversity and crop pollination services are at risk. *Trends in Ecology and Evolution*, 20, 651-651.
- SFO. (2013). *Vallfröarealer*. Sveriges frö- och oljeväxtodlare, Alnarp, Sweden.  
<http://www.svenskraps.se/vallfro>
- Stern, V.M., Smith, R.F., van den Bosch, R. & Hagen, K.S. (1959). The integration of chemical and biological control of the spotted alfalfa aphid. The integrated control concept. *Hilgardia*, 29, 81-101.
- Strauss, S.Y. & Irwin, R.E. (2004). Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution & Systematics*, 35, 435-466.
- Strauss, S.Y. & Murch, P. (2004). Towards an understanding of the mechanisms of tolerance: compensating for herbivore damage by enhancing a mutualism. *Ecological Entomology*, 29, 234-239.
- Taylor, N. L. & Quesenberry, K.H. (1996). *Red clover science*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Tilman, D., Balzer, C., Hill, J. & Befort, B.L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the USA*, 108, 20260-20264.
- Topbjerg, H. & Ytting, N. (2009). *Weevil pests in organic white clover seed production (Trifolium repens)*. Master thesis, University of Copenhagen, Copenhagen, Denmark.
- Tscharntke, T., Bommarco, R., Clough, Y., Crist, T. O., Kleijn, D., Rand, T. A., Tylianakis, J., van Nouhuys, S. & Vidal, S. (2007). Conservation biological control and enemy diversity on a landscape scale. *Biological Control*, 43, 294-309.
- Tscharntke, T., Tylianakis, J., Rand, T., Didham, R., Fahrig, L., Batary, P., Bengtsson, J., Clough, Y., Crist, T., Dormann, C., Ewers, R., Holt, R., Holzschuh, A., Klein, A., Kremen, C., Landis, D., Laurance, B., Lindenmayer, D., Scherber, C., Sodhi, N., Steffan-Dewenter, I., Thies, C., vanderPutten, W., Westphal, C., Fruend, J. & Kleijn, D. (2012a). Landscape moderation of biodiversity patterns and processes – eight hypotheses. *Biological Reviews*, 87, 661-685.
- Tscharntke, T., Clough, Y., Wanger, T.C., Jackson, L., Motzke, I., Perfecto, I., Vandermeer, J. & Whitbread, A. (2012b). Global food security, biodiversity conservation and the future of agricultural intensification. *Biological Conservation*, 151, 53-59.
- Wallace, K.J. (2007). Classification of ecosystem services: problems and solutions. *Biological Conservation*, 139, 235-246.
- Wearing, C.H. (1988). Evaluating the IPM implementation process. *Annual Review of Entomology*, 33, 17-38.
- Williams, P., Colla, S. & Xie, Z. (2009). Bumblebee vulnerability: common correlates of winners and losers across three continents. *Conservation Biology*, 23, 931-940.

- Williams, N.M., Regetz, J. & Kremen, C. (2012). Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. *Ecology*, 93, 1049-1058.
- Whitehorn, P.R., O'Connor, S., Wackers, F.L. & Goulson, D. (2012). Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science*, 336, 351-352.
- Winfree, R., Williams, N.M., Dushoff, J. & Kremen, C. (2007). Native bees provide insurance against ongoing honey bee losses. *Ecology Letters*, 10, 1105-1113.
- Winfree, R., Aguilar, R., Vázquez, D.P., LeBuhn, G. & Aizen, M.A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, 90, 2068-2076.
- Witte, H. (1940). 1939 års inventering av till frötäkt avsedda arealer utav rödklöver, alsikeklöver och timotej. *Svensk Frötidning*, 9, 65-67.
- Zalom, F.G. (1993). Reorganizing to facilitate the development and use of integrated pest management. *Agriculture, Ecosystems & Environment*, 46, 245-256.
- Zalucki, M.P., Adamson, D. & Furlong, M.J. (2009). The future of IPM: whither or wither? *Australian Journal of Entomology*, 48, 85-96.
- Zehnder, G., Gurr, G.M., Kühne, S., Wade, M.R., Wratten, S.D. & Wyss, E. (2007). Arthropod pest management in organic crops. *Annual Review of Entomology*, 52, 57-80.
- Östman, Ö., Ekbom, B. & Bengtsson, J. (2003). Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecological Economics*, 45, 149-158.



## Populärvetenskaplig sammanfattning

Befolkningsökningen och ökat välbefinnande i världen förväntas fördubbla efterfrågan på mat de närmaste årtiondena. Jordbruket har redan genomgått stora förändringar för att möta den ökade efterfrågan på mat, till exempel genom ökad användning av konstgödsel och bekämpningsmedel. Det tidigare mosaikartade landskapet, med en blandning av små åkrar, betesmarker och våtmarker, har i många områden i Europa ersatts av ett likformigt landskap som domineras av intensivt odlad åkermark. Detta har inneburit att många växter och djur i jordbrukslandskapet har förlorat eller fått sina livsmiljöer förstörda. En minskande biologisk mångfald i jordbrukslandskapet kan påverka jordbruksproduktionen negativt, men det saknas information om hur funktionellt viktiga delar av den biologiska mångfalden, som insekter som pollinerar grödor, skadegörande insekter och deras naturliga fiender klarar sig i det moderna jordbrukslandskapet i jämförelse med i äldre tider. Dessutom behövs mer information om hur dessa insektsgrupper påverkar skördarna, och om hur skadliga och nyttiga insekter ska förvaltas i moderna jordbrukssystem. Syftet med denna avhandling har varit att undersöka dessa frågor, och vi har använt fröodling av rödklöver som ett modellsystem för att undersöka detta.

Den nytta som den biologiska mångfalden ger oss människor kallas populärt för ekosystemtjänster. Pollinering och kontroll av skadegörare är viktiga exempel på ekosystemtjänster. 75 procent av de vanligaste grödorna i världen, framförallt olika frukter, grönsaker och oljeväxter, gynnas av insektspollinering. De volymmässigt allra största grödorna, spannmål, majs och ris är däremot inte insektspollinerade. Honungsbiet är en viktig pollinerande insektsart, men även många vilda arter av humlor och bin pollinerar grödor. Flera studier har visat att de vilda pollinatörerna minskar i artrikedom och antal. Förlust och försämrad kvalitet av deras livsmiljöer anses vara den viktigaste orsaken till detta. Än så länge finns det dock få exempel som visat

att detta påverkat skördarna för de insektpollinerade grödorna negativt. Insektsskadegörare å andra sidan gör oss en 'otjänst'; de minskar skördarna med mellan 5 och 15 procent i alla de vanligaste grödorna trots ihärdiga försök att kontrollera dem med framförallt kemiska växtskyddsmedel. I ett historiskt perspektiv har denna trend varit konstant eller till och med ökande. En möjlig förklaring till att skördebortfallen är konstanta eller ökar trots kemisk bekämpning är att moderna odlingslandskap som domineras av åkrar och där användningen av insatsmedel är hög gynnar skadegörarna men missgynnar deras naturliga fiender. På 50- och 60-talen insåg man att kemiska växtskyddsmedel ibland kan ha negativa effekter på kontrollen av skadegörare, och att användningen även ha skadliga effekter på hälsan, på växter och djur samt på miljön. Som en följd utvecklades så kallat integrerat växtskydd, där man försöker kombinera flera olika metoder för att kontrollera skadegörare. Tanken med integrerat växtskydd är att man endast ska använda kemiska växtskyddsmedel när man misslyckas med att kontrollera skadegöraren på andra sätt. För att fortsätta utveckla det integrerade växtskyddet behövs det mer information om vilken skada olika insekter gör och hur de kan kontrolleras med i första hand icke-kemiska metoder, eller i andra hand med effektiva och målinriktade kemiska metoder.

Rödklöver är en viktig fodergröda i jordbruket som dessutom förbättrar markstrukturen och binder kväve från luften som annars är svåråtkomligt för växter. Sverige är en av de större producenterna av rödklöverfrö i Europa med över 2000 hektar fröodling. Betalningen för fröna är hög; cirka 20 till 50 kr per kg, men skördarna varierar enormt mellan 0 och ungefär 800 kg per hektar, vilket gör det till en riskfylld gröda att odla. Rödklöver är helt beroende av insektpollinering för att sätta frö, och utan blombesökande insekter blir det alltså inte några frön alls. Humlor och honungsbin är de vanligaste pollinatörerna. Blommorna är djupa i förhållande till pollinatörernas tunglängd. Därför är det långtungade humlearter som har lättast för att pollinera rödklöver. Jordhumlor som har korta tungor, väljer ibland istället att bita hål på botten av blompiparna för att komma åt nektarn. När det sker överförs inte pollenkornen som humlan har på sin kropp lika bra till blomman och frösättningen uteblir. Rödklöver har som alla grödor även skadegörare och de allvarligaste insektsskadegörarna i rödklöverfröodling är klöverspetsvivlar. Den största skadan görs av vivelns larver som utvecklas inne i blomhuvudena. Varje larv äter 6 till 10 fröanlag. Olika arter av parasitsteklar lägger sina ägg i larverna inuti blomman. Parasitstekelns larver äter upp vivellarven och begränsar på så sätt mängden klöverspetsvivlar. Det vanligaste sättet att kontrollera klöverspetsvivlarna är med kemisk bekämpning. Det finns dock ingen bekämpningströskel som anger vid vilken skadegörarnivå det lönar sig att



bekämpa, och det finns dålig kunskap om alternativa kontrollmetoder samt vilken roll parasitsteklarnas biologiska kontroll spelar.

Vi återinventerade platser i Sverige där man tidigare, under 1940- och 1960-talen, inventerat humlor i rödklöverfröodlingar. Korttungade jordhumlor och stenhumlor som tidigare utgjort 40 procent av alla humlor hade ökat i andel och utgjorde nu 89 procent av alla humlor. Flera andra mer långtungade arter, som klöverhumlan, trädgårdshumlan och åkerhumlan hade gått tillbaka procentuellt och var nu relativt ovanliga i odlingarna. De långtungade humlearterna är generellt mer specialiserade i sitt födoval och en minskad tillgång av deras nektar- och pollenväxter kan vara en förklaring till att de gått tillbaka. Vi samlade också in data över förekomsten av klöverspetsvivlar och deras parasitsteklar i sammanlagt 53 odlingar under 4 år i Skåne och jämförde detta med äldre skånska undersökningar från 60 odlingar under tre år på 1930-talet. Förekomsten av klöverspetsvivlar hade fördubblats samtidigt som parasiteringsgraderna generellt var lägre än det historiska medelvärdet. Genom att sammanställa och analysera observationer av klöverspetsvivlar i Skåne gjorda genom åren kunde vi se att det var den rödbenta klöverspetsviveln som på senare tid tagit över som den vanligaste skadegöraren. Tidigare har det varit den allmänna klöverspetsviveln som varit vanligast. Det finns uppgifter om att den rödbenta klöverspetsviveln trivs bättre på varma och torra platser och därför kan ett förändrat klimat, speciellt varmare vårar, ligga bakom artförskjutningen.

Vi sammanställde också data över de svenska fröskördarna av rödklöver sedan början av 1900-talet och fann att skördarna sedan några årtionden tillbaka varit vikande och också blivit alltmer varierande. Ett pollinatörssamhälle som numera domineras av ett fåtal arter samt ökade förekomster av skadegörare har förmodligen bidragit till detta. En separat analys av de data vi samlat in i fält styrker att förekomsten av skadegörare är en viktig bidragande faktor, eftersom vi kunde visa att mängden klöverspetsvivlar hade en tydlig negativ inverkan på skörden. Ett arbete som återstår är att mer detaljerat undersöka hur förändringar i mängd och sammansättning av pollinatörer påverkar skörden.

För att i detalj studera hur både pollineringen och skadegörarkontrollen påverkar skörden utförde vi ett experiment där rödklöverplantor med antingen låg eller hög förekomst av vivlar pollinerades olika mycket av humlor i burar. Resultaten visade att det endast blev en hög frösättning i plantorna med få vivlar som fått en hög nivå av humlepollinering. Om kontrollen av skadegörare var hög men pollineringen låg, eller tvärtom, blev fröskörden däremot nästan lika låg som i försöksledet där båda ekosystemtjänsterna var på en låg nivå. Studien visar att olika ekosystemtjänster, som pollinering och

skadegörarkontroll, kan påverka och samverka med varandra i sitt bidrag till grödproduktionen.

Vi gjorde flera närmare undersökningar av klöverspetsvivlarna och deras naturliga fiender. Målsättningen var att utveckla det integrerade växtskyddet av skadegörarna. Under 2008 och 2011 anlade vi obesprutade zoner i 29 skånska rödklöverfält. I dessa mätte vi mängden skadegörare, hur stor andel av skadegörarna som parasiterades av naturliga fiender samt fröskördarna. Dessa resultat jämfördes med mätvärden från resten av fältet där odlarna valde hur bekämpningen skulle gå till. En stor skillnad mellan de båda åren var vilka typer av kemiska växtskyddsmedel som användes. Under 2008 använde odlarna olika kemiska bekämpningsmedel av typen pyretroider. Dessa verkar om skadegöraren kommer i direkt kontakt med medlet. Under 2011 däremot användes tiaklopid som är ett bekämpningsmedel av typen neonikotinoider. Detta är en grupp växtskyddsmedel som är systemiska, vilket betyder att de tas upp av växten och gör den giftig för skadegöraren. Det visade sig att pyretroider inte var effektiva eftersom varken skadegörarna minskade eller skördarna ökade på något märkbart sätt av denna behandling. Neonikotinoiden tiaklopid visade sig däremot kunna minska förekomsten av klöverspetsvivlar kraftigt och öka skördarna. I denna studie kunde vi också visa att man kan förutsäga ungefär hur stort skördebortfallet kommer att bli redan i grödans knoppstadium genom att mäta förekomsten av klöverspetsvivlar i fångstskålar som man sätter ut i fältet. Detta är värdefull kunskap eftersom odlaren ofta behöver besluta redan i grödans knoppstadium om kemisk bekämpning ska ske eller ej. Fångstskålarna som vi använde var vanliga djupa plasttallrikar som fylldes till hälften med vatten och lite diskmedel. Eftersom fröpriset är högt och skadan förhållandevis stor per vivel, visade våra uträkningar att det lönar sig att bekämpa mot klöverspetsviveln redan när man fångar mer än en till två vivlar per vecka i skålarna i knoppstadiet. Baserat på resultaten rekommenderar vi därför att man bekämpar med tiaklopid om mängden klöverspetsvivlar överskrider bekämpningströskeln. Neonikotinoider har på senare tid uppmärksamats och satts i samband med bidöd, men detta har gällt andra preparat än tiaklopid som är giftigare för bin och som bryts ned mycket långsammare i naturen. Som en försiktighetsåtgärd anser vi dock att man inte ska spruta tiaklopid i blommande gröda som ibland sker idag, utan istället sent i knoppstadiet. Då kommer halterna av bekämpningsmedlet i klövern förmodligen sjunka ordentligt innan pollinatörerna och parasitsteklarna blir som mest aktiva i grödan.

Vi undersökte även vilka faktorer utöver den kemiska bekämpningen som styr förekomsten av klöverspetsvivlar och parasiteringsgrader. Data till denna undersökning samlades i 53 fält i Skåne under 2008 till 2011. Dessa analyser

visade att förekomsten av klöverspetsvivlar var konsekvent högre i storskaliga jordbrukslandskap med en hög andel åkermark jämfört med i mer brutna odlingslandskap med en lägre andel åkermark. Däremot påverkades inte parasiteringsgraderna av landskapstypen på något tydligt sätt. Ytterligare undersökningar behöver göras för att ta reda på vad som kan ligga bakom detta resultat. Kan klöverbollar, som är vanligare i de brutna odlingslandskapen, fungera som "fällor" och minska mängden klöverspetsvivlar i fröodlingarna? Har klöverspetsvivlarna andra naturliga fiender förutom parasitsteklarna? Stora nederbördsmängder på våren och sommaren var också relaterat till lägre skadegörarförekomster. Slutligen visade det sig att rödklöverfröodlingar där avståndet till föregående års rödklöverfröodling var minst 800 meter hade ungefär hälften så låga förekomster av klöverspetsvivlar jämfört med odlingar där detta avstånd var mindre än 200 meter. Avstånd mellan rödklöverfält från ett år till nästa kan till viss del styras av odlaren i växtföljden och kan därför användas som en strategi för att förebygga att stora skadegörarpopulationer byggs upp.

Sammantaget pekar denna avhandling på att grödpollinering och skadegörarkontroll kan äventyras av odlingsmetoderna i moderna jordbrukslandskap och att detta kan sänka produktiviteten i jordbruket. Den visar vidare att interaktioner mellan olika grupper av funktionellt viktiga insekter kan påverka den nytta som kommer oss till godo i form av ökade skördar. Det behövs därför fler studier som undersöker interaktioner och sammanlagd effekt av de olika ekosystemtjänster som understödjer jordbruksproduktionen. Gynnande av nyttoorganismer som understödjer jordbruksproduktionen och integrerat växtskydd med miljövänliga metoder mot skadegörare kan bidra till hållbar matproduktion i jordbruket.



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